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Effects of Taurine on Lipid Metabolism and Protein Synthesis in Poultry and Mice*

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ABSTRACT : In this study, we have attempted to understand the effects of taurine on serum and liver concentrations of cholesterol and triglycerides in broiler chickens and mice in the post-absorptive state, and on *in vitro* protein synthesis in the livers of broiler chickens and laying hens, as well as the effects of taurine on *in vivo* protein synthesis in the liver of mice. The experimental animals were subjected to 24 h of starvation in order to perpetuate a post-absorptive state. Serum concentrations of high density lipoprotein cholesterol and triglycerides were significantly ($p<0.05$) higher in the taurine groups than in the controls in both the broilers and the mice. However, taurine resulted in a significant ($p<0.05$) reduction in liver concentrations of total cholesterol and triglycerides, relative to what was seen in the control groups of both animals. Taurine stimulated the *in vitro* synthesis of 57-kDa, 40-kDa and 23-kDa proteins in the liver of broilers, but inhibited the *in vitro* synthesis of 54-kDa, 37-kDa and 24-kDa proteins. Taurine in the liver of laying hens exerted effects on *in vitro* protein synthesis, with the exception of the 26-kDa protein which was not detected in broiler liver, but was inhibited by taurine in the liver of laying hens. Unlike the findings regarding *in vitro* protein synthesis in the liver of broilers or laying hens, taurine appeared to stimulate the synthesis of only two proteins, a 47-kDa and a 40-kDa protein, in the liver of mice. Overall, theses findings indicate that taurine treatment results in a reduction in cholesterol and triglyceride concentrations, and also affects protein synthesis in the livers of broilers, laying hens, and mice. (**Key Words :** Taurine, Lipid Metabolism, Protein Synthesis, Broilers, Laying Hens, Mice)

INTRODUCTION

Since Tiedemann and Gmelin (1827) initially identified taurine in ox bile, a great deal of attention has been focused on the biological functions of taurine. Taurine (2-aminoethanesulfonic acid) can be detected in many animal tissues, existing principally as a free amino acid, but is not involved in protein synthesis, nor is it employed as an energy source in animals. Although taurine has been reported to perform functions in membrane stabilization (Pasantes-Morales et al., 1985), anti-oxidation (Nakamura et al., 1993; Jang et al., 2008), osmo-regulation (Thurston et al., 1980), detoxification (Huxtable, 1992), neuro-modulation (Kuriyama, 1980; Bernardi, 1985) and brain and retinal development (Sturman, 1986), the most well-established function of taurine is the conjugation of bile acids in the liver, resulting in the formation of water-soluble bile salts which are crucial to micelle formation with lipids and thereby effecting an overall increase in fat absorption within the small intestine (Tanno et al., 1989; Catafora et al., 1991). For example, Colombo et al. (1988) previously reported that taurine effects an increase in lipid absorption, as well as increasing serum triglyceride concentrations and inducing weight gain in children suffering from cystic fibrosis involving ongoing fat malabsorption.

Taurine also performs pivotal functions in lipid metabolism. Numerous studies have demonstrated the effects of taurine supplementation on plasma and/or liver lipid concentrations in different animal species. The results of these studies tend to vary depending on the species of animal used, the term of taurine supplementation, and the composition of the experimental diet. For example, taurine supplementation was shown to augment liver triglyceride and cholesterol levels in guinea pigs (Cantafora et al., 1986)

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and cats (Cantafora et al., 1991), but resulted in a decrease in these levels in rats (Gandhi et al., 1992; Yan et al., 1993), mice (Yamanaka et al., 1985) and Japanese quail (Jeckson and Burns, 1974) fed on a normal diet. Our previous studies demonstrated that the effects of taurine on the concentrations of lipids in the livers of broilers or laying hens tend to differ in accordance with the amount of taurine supplementation administered (Park and Choi, 1997; Kim and Park, 2002; Park, 2002; Shim et al., 2006). The differential effects of taurine on liver lipid concentrations have yet to be clearly elucidated and assigned practical and scientific meaning, in that the quantities of lipids within the liver rely on the rate of lipid metabolism in the liver as well as the absorption rate of lipids via the small intestine. Therefore, further studies in the post-absorptive state are clearly necessary in order to determine the direct effects of taurine on lipid metabolism in the liver, without contending with the increase in lipid absorption associated with taurine treatment. Also, changes in lipid metabolism in the liver may be related to protein synthesis in the liver. Therefore, the primary objective of this study was to determine the direct effects of taurine injection on serum and liver concentrations of lipid fractions in broiler chickens and mice in the post-absorptive state. Furthermore, effects of taurine on *in vitro* protein synthesis in the livers of broiler chickens and laying hens were determined, as well as effects on *in vivo* protein synthesis in the liver of mice.

MATERIALS AND METHODS

Animals and management

In this study, we used three experimental animals: male broiler chickens (Cobb), mice (ICR), and laying hens (ISA Brown). Day-old male broilers were obtained, kept in electrically-heated battery brooders and raised on commercial starter diets (Agribrands Purina Korea Inc., Korea) up to 41 days of age. Ambient temperature was gradually reduced from 32°C at one day of age, to 22°C at the final age of 41 days. Water was available *ad libitum*, and continuous lighting was applied. Mice, acquired from Damul Science (Yoosung, Korea) were maintained in individual cages at 22°C with a 12/12-h light/dark photocycle (lights on at 08:00 h), and were provided free access to water. They also had free access to Samyang feed stuff laboratory pellets (Samyang, Co., Korea). The laying hens (13 months old; 1,900-2,200 g BW) were obtained from the Jinis Biopharmaceutical Company (Jeonju, Korea). They were housed in electrically-heated battery brooders, and raised on an identical diet to that of the broilers. The hens were permitted to acclimatise to the new environment for 7 days, with free access provided to both diet and water. The lights were maintained for 18 h a day. The Animal Care and Use Committee of Chonbuk National University approved the experimental protocol.

Taurine injection

Feed was taken away from all animals 24 h prior to the initiation of the treatments. The 10 male broiler chickens and 20 male mice were divided into two groups each, with similar body weights of 2,100-2,400 g and 30-35 g, respectively. In the studies designed to determine the effects of taurine on lipid fractions in the serum and liver, the male broiler chickens were injected in the wing vein and male mice were injected in the tail vein with taurine in a saline solution. The taurine group was injected with 0.15 mg and 0.33 mg of taurine per 100 g of body weight in the broiler chickens and mice, respectively. The control animals received injections consisting of an equal amount of saline solution. All solutions were prepared with distilled water and filtered through a 0.22 μm membrane.

Blood and liver sampling

Exactly two hours after the injections of each of the aforementioned solutions, the animals were decapitated, and blood was collected, allowed to clot, and then centrifuged in order to obtain the serum fractions. Liver samples were quickly excised, then washed in cold 0.9% NaCl solution. The serum and liver samples were kept in a freezer at -20°C until analysis.

Serum and liver lipid analysis

Serum concentrations of total cholesterol, high density lipoprotein (HDL) cholesterol, and triglycerides were determined using analytical kits purchased from Asan Pharm. Co., Ltd. (Seoul, Korea). Extractions of total lipids from the liver were conducted as described in the study of Folch et al. (1957). The contents of extracted lipids were estimated as described in Bligh and Dyer (1959). The cholesterol or triglyceride concentrations in the lipids extracted from the liver were also enzymatically determined, as described for plasma cholesterol and triglyceride concentrations.

In vitro protein synthesis

The 10 broiler chickens and 8 laying hens were divided into two groups. After 24 h of starvation, the experimental animals were slaughtered via neck dislocation, and samples of each liver were extracted into ice-cold Dulbecco's Modified Eagle's Medium (DMEM) (Sigma-Aldrich Inc., St. Louis, MO 63103 USA) and immediately prepared for labeling as previously described by Yahav et al. (1997). In brief, the liver samples were sliced to an approximate thickness of 20 mg, washed in DMEM, and then incubated in individual tubes. To each tube, 200 μCi of [^{35}S]-methionine (1,175 Ci/mmol, PerkinElmer Life Science, Inc., Boston, MA 02118) was added in 500 μl DMEM containing

0 to 120 μM taurine. The slices were then incubated for 45 minutes in a shaker at 41°C. The tissues were homogenized with a glass bar and sonicated for 30 seconds in ice, a procedure that was repeated 3 times. The homogenates were centrifuged for 30 minutes at 15,000 g at 4°C in order to remove any cellular debris. The resulting supernatants were re-centrifuged for 90 minutes at 15,000×g at 4°C, after which the supernatant fractions were stored at -20°C for electrophoretic analysis.

***In vivo* protein synthesis**

In order to determine the effects of taurine on protein synthesis within the liver, the mice were injected intravenously with taurine and [^{35}S]-methionine (1,175 Ci/mmol, Perkin-Elmer Life Sciences, Inc., Boston, MA 02118) in saline solution. The experimental animals were fasted for 24 h and divided into two groups. The taurine group of mice (n = 4) was injected with 0.33 mg of taurine and 3.3 mCi [^{35}S]-methionine per 100 g of body weight. The control group (n = 4) received injections of 3.3 mCi [^{35}S]-methionine in saline solution, rather than taurine. Two hours after exposure, the animals were slaughtered via decapitation and the blood was collected, allowed to clot, and then centrifuged to obtain the serum fraction. The liver samples were quickly extracted, washed in cold 0.9% NaCl solution, and homogenized in 5 volumes of phosphate-buffered saline (PBS; 16 mM Na_2HPO_4, 1 mM NaH_2PO_4, 140 mM NaCl, and pH 7.4) for 30 seconds in ice, and this procedure was repeated 3 times. The homogenates were centrifuged for 30 minutes at 2,000×g at 4°C in order to remove any cellular debris. The resultant supernatant was then recentrifuged for 90 minutes at 15,000×g and 4°C, after which the supernatant fraction was stored at -20°C for electrophoretic analysis.

Measurement of radioactivity

The radioactivity of the cellular proteins from the *in vivo* and *in vitro* trials was measured as previously described (Yahav et al., 1997). In brief, 5 μl aliquots of the supernatant from each sample were spotted onto nitrocellulose filters, either in duplicate or triplicate. The cellular proteins were precipitated on the filters via 15 minutes of incubation at 4°C in 10% ice-cold trichloroacetic acid (TCA), boiled for 10 minutes in 5% TCA, washed in ethanol, and counted using a liquid scintillation counter (Digital LSC, Wallac Co., Finland).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and autoradiography

The cellular proteins were separated via one-dimensional 10% SDS-PAGE (Laemmli, 1970) using a large-slab gel apparatus (Hopper model SE 600, Amersham Pharmacia Biotech). The protein samples were mixed with equal volumes of 2 sample loading buffer (125 mM tris-HCl, pH 6.8, 20% glycerol, 2% SDS, 2% 2-mercaptoethanol, and 1% bromophenol blue) and heated for 5 minutes to a temperature of 95°C. Each of the samples was loaded onto 10% SDS-polyacrylamide gel (10% separating gel, pH 8.8 overlaid with 4% stacking gel, at a pH of 6.8) to ensure equal amounts of radioactivity for each lane. Electrophoresis was then conducted with a constant current of 20 mA per gel at room temperature for approximately 5 h, until the bromophenol blue arrived at the bottom of the gel. The gel was stained with coomassie brilliant blue staining solution (0.05% coomassie brilliant blue R250, 50% methanol, and 10% acetic acid) and de-stained with de-staining solution (25% methanol and 7.5% acetic acid). The gel was then dried in a drying oven for 24 h at 30°C, and exposed to autoradiography using BAS 1000 film (Fuji Film, Fuji Photo Film Co., Japan) for approximately 3-7 days. The signal intensities of each of the bands appearing on the film were then scanned via digital autoradiography (BAS 1000, Fuji Photo Film Co., Japan) and quantified with an image analyzer (Imagemaster 1D Prime, Amersham Pharmacia Biotech) within the log curve of a standard curve. The background was then subtracted from each band. The following molecular standard proteins (Low Molecular Weight Electrophoresis Calibration Kit, Amersham Pharmacia Biotech) were used: phosphorylase B (94 kDa), bovine serum albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20.1 kDa) and α-lactalbumin (14.4 kDa).

Statistical analysis

All experiments were simultaneously conducted, in order to eliminate variances resulting from the preparation, storage, and handling of samples at different times. The experimental data were analyzed with the SAS (1999) statistical program. Data were analyzed with Student's t-tests in order to determine the significance of treatment effects (Huntsberger and Billingsley, 1981).

RESULTS AND DISCUSSION

The effects of taurine injection on cholesterol and triglyceride in the sera and liver samples of broiler chickens and mice in the post-absorptive state are shown in Table 1 and 2. In our study, the animals were subjected to starvation for 24 h, in order to perpetuate a post-absorptive state. In both species, serum concentrations of HDL-cholesterol and triglycerides were significantly ($p<0.05$) higher in the taurine groups than in the controls. However, the injection of taurine was found to significantly ($p<0.05$) reduce the concentrations of total cholesterol and triglycerides in the livers of the experimental animals, relative to the levels measured in the controls. One of the best-characterized

Table 1. Effects 4of taurine injection on the lipid fractions of broiler serum and liver[1]

Items	Saline	Taurine[2]
Serum		
Total cholesterol (mg/dl)	123±6.42	123±3.44
HDL-cholesterol (mg/dl)	45±2.03[b]	52±2.16[a]
Triglyceride (mg/dl)	24.5±1.72[b]	40.1±3.65[a]
Liver		
Total cholesterol (mg/g)	2.08±0.77[a]	1.70±0.76[b]
Triglyceride (mg/g)	13.5±0.43[a]	11.9±0.64[b]

[1] Mean±SE.
[a, b] Means in a row with no common superscripts differ significantly ($p<0.05$).
[2] Taurine group was injected with 0.15 mg of taurine per 100 g of body weight.

Table 2. Effects of taurine injection on the lipid fractions of mice serum and liver[1]

Items	Saline	Taurine[2]
Serum		
Total cholesterol (mg/dl)	129±5.95	116±4.81
HDL-cholesterol (mg/dl)	80±2.87[b]	106±11.24[a]
Triglyceride (mg/dl)	46±1.05[b]	60±5.18[a]
Liver		
Total cholesterol (mg/g)	7.39±0.29[a]	5.89±0.39[b]
Triglyceride (mg/g)	16.1±2.09[a]	10.8±1.30[b]

[1] Mean±SE.
[a, b] Means in a row with no common superscripts differ significantly ($p<0.05$).
[2] Taurine group was injected with 0.33 mg of taurine per 100 g of body weight.

functions of HDL is the promotion of the removal of cholesterol from the cells. Therefore, our data, which revealed an increase in the serum concentrations of HDL-cholesterol and a reduction in the concentrations of total cholesterol in the liver as a result of taurine injection, also indicated that taurine may promote the transport of cholesterol to the liver from other tissues, the degradation of cholesterol in the liver, and the excretion of cholesterol from the liver to the small intestine via the gall bladder. This hypothesis is bolstered by the findings of Yokogoshi et al. (1999). Kerai et al. (1999) also reported that taurine stimulates the secretion of triglycerides from the liver of rats. Our data also indicated that taurine injection increased serum triglyceride concentrations, and reduced triglyceride concentration in the livers of broilers as well as mice, thereby indicating that taurine can stimulate the secretion of triglycerides from the liver to the blood in both species.

The changes in cholesterol and triglyceride concentrations in the liver observed in this study may be associated with protein synthesis in the liver. As shown in Figure 1 and Table 3, taurine stimulated the *in vitro* synthesis of a new protein at 57-kDa and significantly ($p<0.05$) increased the synthesis of the 40-kDa and 23-kDa proteins in the liver samples obtained from the broilers. Concurrently, taurine decreased ($p<0.05$) the *in vitro* synthesis of the 54-kDa and 37-kDa proteins and completely inhibited synthesis of the 24-kDa protein in the liver of broilers. Taurine in laying hens exerted similar effects on stimulation and inhibition of *in vitro* protein synthesis as seen in the liver of broilers ($p<0.05$), with the exception of the 26-kDa protein, which was not detected in broiler liver samples, but was shown to have been inhibited by taurine in the liver of laying hens (Figure 2 and Table 4).

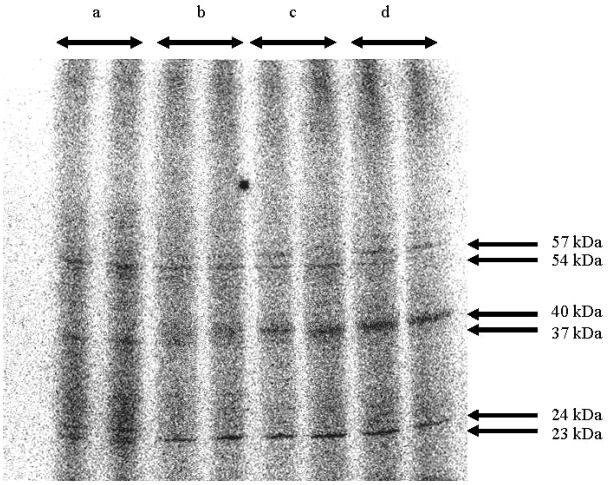

Figure 1. Autoradiogram of newly synthesized proteins in liver samples of broiler chickens *in vitro*. a. Control; b. 120 μM taurine levels; C; 240 μM taurine levels; d: 480 μM taurine levels.

Table 3. The effect of taurine on *in vitro* protein synthesis in liver samples of broiler chickens[1]

Protein molecular weight (kDa)	Band intensity (%)[2]			
	Control	Taurine		
		120 μM	240 μM	480 μM
57	ND[3]	1.23±0.34	2.20±0.80	2.26±0.14
54	4.10±0.76[a]	3.70±0.53[a]	3.04±0.24[ab]	1.40±0.04[b]
40	1.73±0.28[c]	5.34±1.04[b]	8.92±0.13[a]	9.98±0.03[a]
37	5.54±0.40[a]	3.48±0.57[ab]	3.05±0.33[b]	2.00±0.79[b]
24	5.28±1.32	ND	ND	ND
23	1.98±0.12[b]	5.33±0.09[a]	5.98±0.08[a]	6.62±0.65[a]

[1] Mean±SE. [2] Band intensity of the autoradiogram shown in Figure 1, are arbitrary units given by image analyzer. [3] ND = Not detected.
[a, b, c] Means in a row with no common superscripts differ significantly ($p<0.05$).

Table 4. The effect of taurine on *in vitro* protein synthesis in liver samples of laying hens[1]

Protein molecular weight (kDa)	Band intensity (%)[2]	
	Control	480 μM Taurine
57	2.87±0.61[b]	10.1±1.77[a]
54	6.43±0.70[a]	3.24±0.29[b]
40	5.59±0.74[b]	15.1±1.86[a]
37	6.83±0.51	ND[3]
26	2.57±0.23	ND
24	2.27±0.16	ND
23	4.02±0.39[b]	7.90±0.99[a]

[1] Mean±SE.
[2] Band intensity of the autoradiogram shown in Figure 2, are arbitrary units given by image analyzer.
[3] ND = Not detected.
[a, b] Means in a row with no common superscripts differ significantly ($p<0.05$).

Table 5. The effect of taurine on *in vivo* protein synthesis in liver samples of mice[1]

Protein molecular weight (kDa)	Band intensity (%)[2]	
	Control	Taurine[3]
57	4.50±0.31	5.26±0.63
54	3.81±0.56	4.22±0.11
47	7.35±0.26[a]	10.3±0.34[b]
40	6.51±0.15[a]	9.06±0.24[b]
37	1.80±0.12	2.07±0.10
23	5.23±0.58	6.37±0.25

[1] Mean±SE.
[2] Band intensity of the autoradiogram shown in Figure 3, are arbitrary units given by image analyzer.
[3] Taurine group was injected with 0.33 mg of taurine per 100 g of body weight.
[a, b] Means in a row with no common superscripts differ significantly ($p<0.05$).

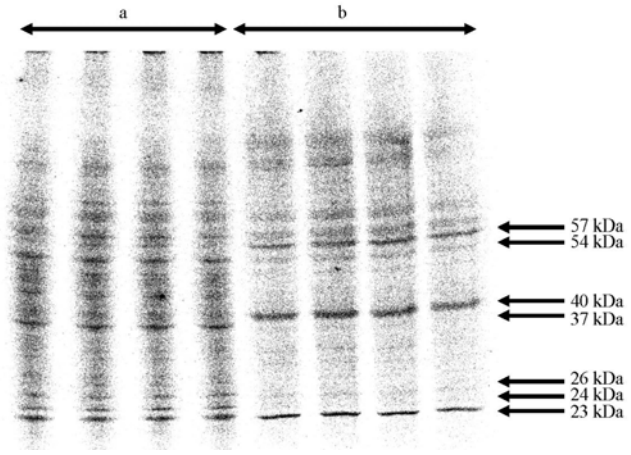

Figure 2. Autoradiogram of newly synthesized proteins in liver samples of laying hens *in vitro*. a. Control; b. 480 μM taurine levels.

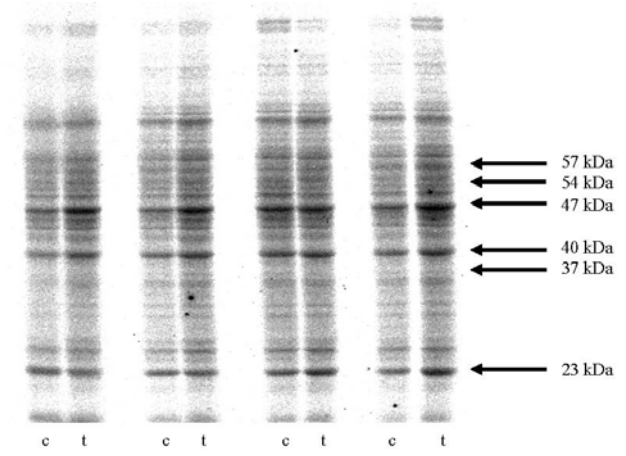

Figure 3. Autoradiogram of newly synthesized proteins in liver samples of mice *in vivo*. c. Control; t. Taurine treatments; mice were injected with 0.33 mg of taurine per 100 g of body weight.

An investigation of *in vivo* protein synthesis in broiler livers would not be feasible, due primarily to the huge amount of isotope that would be necessary for an *in vivo* study in chickens. Thus, effects of taurine injection on *in vivo* protein synthesis was determined using the liver of mice, as the effects of taurine on serum and liver lipid concentrations should be quite similar between the two species. The results of these trials are shown in Figure 3 and Table 5. Unlike the results regarding *in vitro* protein synthesis in the liver of the broilers or the laying hens, taurine stimulated ($p<0.05$) the synthesis of only two proteins, a 47-kDa and a 40-kDa protein, in the liver of mice.

In conclusion, our data clearly show that taurine effects a reduction in the concentrations of both cholesterol and triglycerides, and also exerts a profound effect on protein synthesis in the liver of both broilers and mice. However, further study will be necessary in order to correctly characterize the effects of taurine on liver proteins, thereby delineating their functional relationships with hepatic cholesterol and triglyceride concentrations in both broilers and mice.

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